The effect of Cd^{2+} on the release of proteins from thylakoid membranes of tomato leaves

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Abstract

The polypeptide pattern of the thylakoid membrane was investigated to elucidate the decrease of photosystem II (PSII) activity due to Cd^{2+} incubation. The release of polypeptides depended on the concentration of the metal and time of incubation. The complete release of the 33 kDa polypeptide and partial release of 18, 23 kDa and 1 atom of Mn/400 chlorophyll indicated some disorganization of the water splitting system. Partial removal of numerous polypeptides, especially that of the PSII complex and coupling factor (CF_0, CF_1), was additionally observed. The Cd^{2+}-induced release of thylakoid membrane proteins was not only limited to the PSII donor side, however the latter was particularly sensitive to Cd^{2+}.

Key words: cadmium, Lycopersicum esculentum Mill., 33 kDa protein, photosystem II activity, photosystem II particles, thylakoid membrane polypeptides

INTRODUCTION

High toxicity of Cd^{2+} for plants was shown in recent years, but the mechanism of its action has not been fully investigated. One of the biochemical effects induced by Cd^{2+} is the inhibition of photosynthetic electron transport. Cd^{2+} inhibits photosystem II (PSII) activity on its donor side, affecting the water splitting system (Bazzaz and Govindjee 1974, Van Duijven-dijk-Matteoli and Desmet 1975). Only Li and Miles (1975) have considered Cd^{2+} to affect the reaction centre of PSII.

De Filippis (1979) and Strickland et al. (1979) suggested that Cd^{2+} toxicity may be connected with membrane permeability; however, no data indicating a correlation between this unequivocal toxicity and membrane damage are available. Recently, Roynet et al. (1981) reported a possible inhibition of electron transport by Cd^{2+} due to thylakoid membrane pertur-bation induced by the metal during greening of barley seedlings.

The results of in vivo experiments with chloroplasts of tomato plants growing in Cd^{2+}-containing medium indicated that inhibition of electron transport around PSII is accompanied by disorganization of the inner structure of chloroplasts, mainly that of grana stacks (Baszyński et al. 1980). A distinct decrease in the content of acyl lipids in thylakoid membranes incubated with Cd^{2+}, or in membranes isolated from chloroplasts of Cd^{2+}-treated plants, as well as the release of fatty acids pointed to impairment of the membrane functions by these lipids, including PSII activity (Krupa and Baszyński 1985, Baszyński 1986). Also, proteins were released by incubation of thylakoid membranes with Cd^{2+} (Krupa and Baszyński 1985). During greening of radish seedlings, Cd^{2+} caused changes in the relative ratios of thylakoid membrane polypeptides (Krupa et al. 1987). The purpose of the present studies was an attempt at answering the question whether photosynthesis inhibition caused by Cd^{2+} is related to the disorganization of the donor side of PSII.

MATERIAL AND METHODS

Thylakoid membranes were isolated from tomato leaves (*Lycopersicum esculentum* Mill. var. New Yorker) by the method of Wild et al. (1980). They were incubated at 20°C with 1-20 µmoles Cd^{2+} per 100 µg of chlorophyll (Chl), and after 0.5-4 hrs pelleted at 25,000 ×g for 25 min. An identical procedure but without Cd^{2+} was used for the control. The supernatant was dialysed and then concentrated to 1 cm³ by ultrafiltration on a Diaflo YM10 membrane (Amicon 8 MC) and purified chromatographically on Sephadex G-75.

The PSII complex was obtained by the method of Kuwabara and Murata (1982a) using a Triton X-100 to Chl ratio of 10:1 (w/w). To release proteins of the water splitting system the PSII complex was incubated with 0.8 M Tris-HCl buffer (pH 8.6) for 20 min and centrifuged at 26,000×g for 30 min.

SDS-PAGE. Electrophoretic separation of proteins of thylakoid membranes was carried out in Laemmli's buffer system (1970) in two ways. The first made use of tube gel electrophoresis with a 6% stacking gel and 12.5% resolving gel. After 2 min of sodium dodecyl sulfate (SDS) solubilization of membranes at 100°C, samples were loaded onto gels and electrophoresis was performed at 4°C at a constant current of 3 mA per tube. The gels were stained with 0.25% Coomassie R-250 blue in 50% methanol and 10% acetic acid, destained in 5% methanol and 7.5% acetic acid. In the second, polypeptides were separated on a 10-20% acrylamide gradient slab gels containing 4 M urea and a 5-16% sucrose gradient at a constant current of 12 mA; a 5% stacking gel was used in this case. Membrane samples were solubilized for 1 hr at room temperature in 60 mM Tris-HCl buffer (pH 7.8), 2% SDS, 1 mM EDTA, 12% sucrose and 0.06 M dithiothreitol with an SDS/Chl weight ratio of 15:1 for

membranes, whereas in the case of the supernatant the SDS/protein ratio was 10:1 (w/w). The gels were stained with 0.1% Coomassie R-250 blue in 40% methanol and 10% acetic acid, and destained in methanol/acetic acid/water (4:1.5:4.5, v/v). Gels were scanned at 600 nm on a Vitatron TLD-100 densitometer. A Pharmacia Fine Chemicals LMW Protein Kit was used as the protein standard for determination of apparent molecular weights. Protein concentration was determined according to Bennett (1982).

Photosynthetic O$_2$ evolution was measured with a Clark type oxygen electrode at 24°C using H$_2$O and ferricyanide as an electron donor and acceptor system. The reaction mixture for measuring O$_2$ evolution contained the following components, in μmol: Hepes-NaOH − 50, NaCl − 100, MgCl$_2$ − 5, NH$_4$Cl − 5, K$_3$Fe(CN)$_6$ − 0.5; chloroplasts equivalent to 25 μg of Chl; final volume 1.9 cm^3. Where indicated, 1.5 μmol 1,5-diphenyl-carbohydrazide (DPC) was used as an alternative electron donor. The reaction mixture was incubated under a red radiation flux of 250 Wm^{-2}. A red filter (Balzer K6) was placed between the actinic radiation source and the reaction chamber.

Chl content was determined according to Arnon (1949).

Mn determination was performed on a Pye Unicam SP-9 atomic absorption spectrophotometer after digestion of samples in a HNO$_3$ and HClO$_4$ mixture (3:1, v/v).

RESULTS

Two-hour incubation of thylakoid membranes with Cd^{2+} caused changes in the amount of membrane proteins depending on Cd^{2+} concentration (Fig. 1). The metal used at concentrations up to 10 μmoles Cd^{2+} per 100 μg of Chl caused a gradual release of protein to over 19% in relation to control,
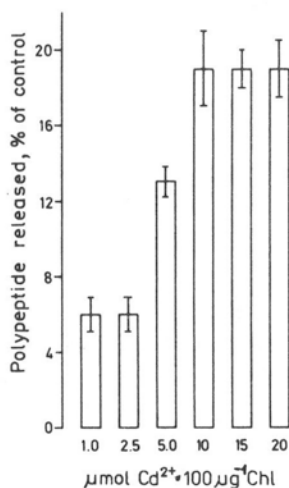

Fig. 1. The effect of Cd^{2+} on the release of protein from thylakoid membranes of tomato leaves after 2 h incubation

which did not change at higher Cd^{2+} doses (up to 20 μmoles). After 2 hr of incubation in the buffer without Cd^{2+} (control samples) about 2.5% of protein was released.

The amount of proteins released from membranes depended on the incubation time (Fig. 2). A level of the proteins released after 2.5 hr incubation at concentration of 10 μmoles Cd^{2+} per 100 μg Chl was about 24% of the control and remained stable for a next 2 hr incubation. Incubation in the buffer without Cd^{2+} resulted in the release of about 5% of the protein after 4.5 hrs.
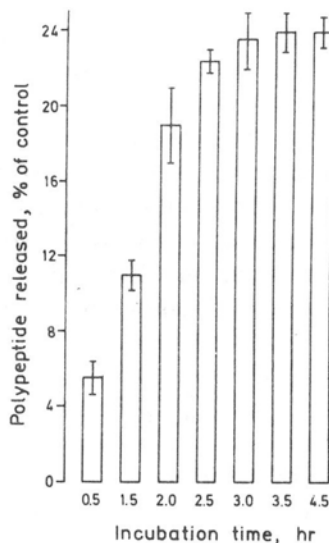

Fig. 2. Effect of incubation time on the release of proteins from thylakoid membranes treated with 10 μmoles Cd^{2+} per 100 μg Chl

The polypeptide pattern of thylakoid membranes incubated with Cd^{2+} showed the total release of the 33 kDa protein and partial release of a considerable number of other polypeptides (Fig. 3). The polypeptides of the water splitting system washed from the PSII complex of tomato leaves with 0.8 M Tris-HCl (pH 8.6) and used as a markers, confirmed the complete release of the 33 kDa polypeptide. The apparent molecular weight of the 33 kDa polypeptide was determined electrophoretically in a system containing 4 M urea; without urea it was estimated as 32 kDa (Fig. 4). The supernatant of Cd^{2+}-incubated thylakoid membranes showed the occurrence of the following polypeptides: 12.5, 13, 18, 23, 24, 32, 33, 36, 37, 40, 47, 59 and 67 kDa (Fig. 5). It is probable that the amount of the released proteins was higher, as the applied purification and concentration of the supernatant may cause protein losses due to binding with Cd^{2+}. It has been shown that about 11% of Cd^{2+} is

strongly bound with proteins; the metal was difficult to remove during dialysis in the presence of EDTA (data not presented).

Incubation of thylakoid membranes with 10 μmol Cd^{2+} per 100 μg Chl decreased the PSII activity found previously. Under experimental conditions the inhibition of photosynthetic O_2 evolution reached 50% of the initial value (Table 1). The addition of DPC, an artificial electron donor, to the reaction

Table 1

The effect of Cd^{2+} on photosynthetic oxygen evolution in thylakoid membranes of tomato leaves

	Control	Cd^{2+}
	μmol $O_2 \cdot mg^{-1}$ Chl$\cdot h^{-1}$	
$H_2O \rightarrow Fe(CN)_6$	139 (100)	71 (51)
$DPC \rightarrow Fe(CN)_6$	142 (100)	107 (75)

Thylakoid membranes were incubated for 2 h with Cd^{2+} ions at a concentration of 10 μmoles Cd^{2+} per 100 μg Chl.
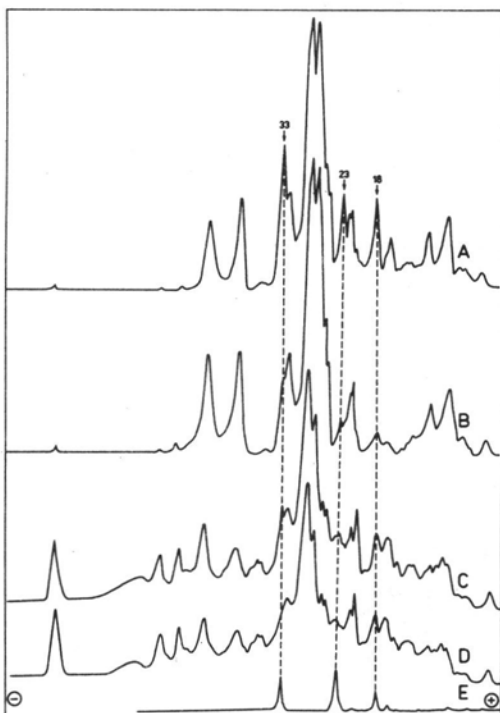

Fig. 3. Polypeptide pattern of thylakoid membranes and PSII complex. A — PSII complex, B — PSII complex washed with 0.8 M Tris-HCl, C — thylakoid membranes, D — Cd²⁺-treated thylakoid membranes, E — supernatant of 0.8 M Tris-HCl-washed PSII complex
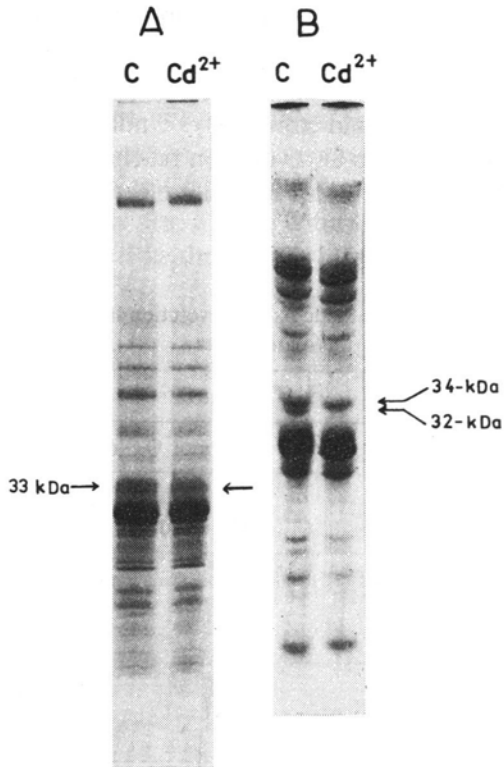
Fig. 4. Polypeptide patterns of control and Cd^{2+}-treated thylakoid membranes. A — urea containing gel, B — gel without urea
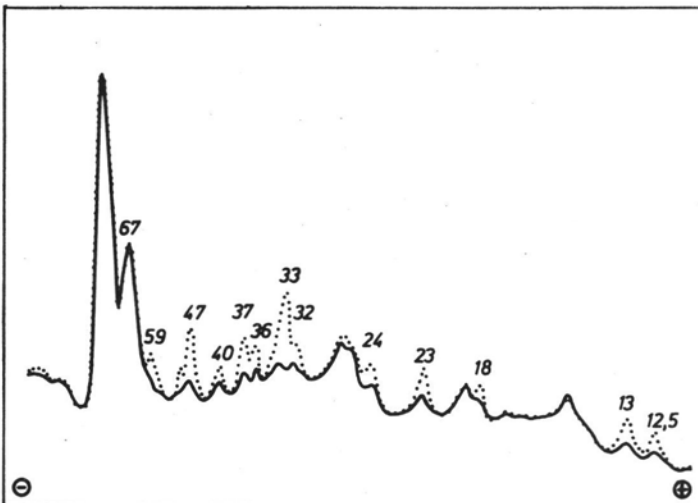

Fig. 5. Polypeptides released from thylakoid membranes of tomato leaves during incubation with Cd^{2+}. Control — solid line; Cd^{2+}-treated — dotted line. Incubation conditions as in Table 1

mixture only partially reversed the inhibitory action of Cd^{2+}. The Cd^{2+}-incubated thylakoid membranes contained 4.2 ± 0.5 Mn/400 Chl, as calculated from atomic absorption spectroscopy measurements. The control preparations not subjected to Cd^{2+} incubation contained 5.3 ± 0.6 Mn/400 Chl (Table 2), Thus, incubation of the membranes with Cd^{2+} was accomplished with removal of only a small part of the metal in relation to the amounts released from 0.8 M Tris-HCl-washed membranes. This last treatment, however, was accompanied by removal of all polypeptides of the water splitting system, i.e. 18, 23 and 33 kDa.

Table 2

Manganese content in Cd^{2+}-treated thylakoid membranes. Values represent the mean $\pm$ SE of three replicates

Thylakoid membranes	Mn/400 Chl
Control	5.3 ± 0.6
+ Cd^{2+}	4.2 ± 0.5
+ 0.8 M Tris (pH 8.6)	2.5 ± 0.3

Incubation conditions as in Table 1.

DISCUSSION

The release of proteins from thylakoid membranes found during their incubation with Cd^{2+} confirmed previous observations (Krupa and Baszyński 1985). The results obtained support the suggested action of Cd^{2+} on photosynthetic electron transport related to disorganization of the water splitting system (see Baszyński 1986). Total release of 33 kDa extrinsic protein unambiguously indicates disruption of this system by Cd^{2+}. This protein, besides 15-18 and 23-24 kDa, is associated with the function of the donor side of PSII (Kuwabara and Murata 1982a, b, Ono and Inoue 1983, Abramowicz and Dismukes 1984, Miyao and Murata 1985, Murphy 1986). The apparent molecular weight of the protein of the manganese system has been determined with urea and without urea as 33 and 32 kDa, respectively (Kyle et al. 1983). A similar behaviour in the presence of urea has been observed in this paper. Hence it seems that protein totally released by the action of Cd^{2+} should be considered as a component of the water splitting system. This was also indicated by the comparison of the molecular weight of this protein with that of the proteins washed with 0.8 M Tris-HCl buffer from the PSII preparation. The destruction of the PSII donor site during incubation with Cd^{2+} was also indicated by a partial release of 18 and 23 kDa polypeptides and Mn^{2+} ions.

Despite the total release of the 33 kDa polypeptide the incomplete loss of thylakoid membrane ability to evolve O_2 might be evidence of its limited role in water photolysis, particularly if a part of the 18 and 23 kDa peptides was not removed from the membrane. This suggestion does not limit the role of the 33 kDa polypeptide necessary for full activity of water oxidation as postulated previously (Miyao and Murata 1984, Tang and Satoh 1986). Attention was also drawn by the loss of only 1 Mn atom during total release of 33 kDa polypeptide (this polypeptide seemed to interact usually with two Mn atoms in the O_2 evolution system (Miyao and Murata 1984)), which indicates indirect involvement of the polypeptide in binding the Mn complex of PSII.

Incomplete restoration of PSII activity in the presence of DPC indicates partial damage of the PSII reaction centre. This site of Cd^{2+} action in the photosynthetic electron transport chain had been taken into consideration earlier (Li and Miles 1975). Partial release under Cd^{2+} treatment of a 47 kDa polypeptide and to a lesser extent of a 40 kDa of the PSII reaction centre accounted for this opinion. Also partial dissociation of 12.5, 13, 37 and 59 kDa polypeptides constituting ATP subunits: II CF_0 and ε, γ, α CF_1 (Nelson 1976, 1981) indicated the known inhibitory effect of Cd^{2+} on non-cyclic and cyclic photophosphorylation (Lucero et al. 1976, Baszyński et al. 1980).

Incubation of thylakoid membranes with Cd^{2+} ions inducing the release of numerous polypeptides revealed a more general character of membrane damage by this metal, which was not limited only to the PSII donor side. However, the total release of the 33 kDa and of a part of the 18 and 23 kDa polypeptides, as well as manganese supported the previous opinion that the water splitting complex is particularly labile in the presence of Cd^{2+}.

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Wpływ Cd^{2+} na uwalnianie białek z błon tylakoidowych liści pomidora

Streszczenie

W celu wyjaśnienia przyczyn spadku aktywności II układu fotosyntezy (PSII) w błonach tylakoidowych inkubowanych z Cd^{2+} badano ich skład peptydowy. Wykazano, że inkubacja błon powoduje uwalnianie białek zależne od stężenia Cd^{2+} i od czasu inkubacji. Całkowite uwalnianie polipeptydu 33 kDa, zaś częściowe 18 i 23 kDa, oraz 1 atomu Mn/400 Chl dowodzi dezorganizacji kompleksu rozszczepiającego wodę. Stwierdzono ponadto częściowe uwalnianie innych polipeptydów, zwłaszcza należących do kompleksu PSII oraz do czynnika sprzęgającego (CF_0, CF_1). Indukowane Cd^{2+} odszczepianie polipeptydów z błony tylakoidowej nie ogranicza się jedynie do układu rozszczepiającego wodę, aczkolwiek układ ten jest szczególnie wrażliwy na Cd^{2+}.